Medical Progress

Biochemistry and Mental States

SEYMOUR S. KETY, M.D., *Boston*

THE SCIENCE OF neurochemistry has grown rapidly in the past decade or two. So great have been the contributions of biochemistry and molecular biology to understanding the chemistry of the brain that they could hardly be covered in the course of an evening's lecture. If we limit the review to those areas of biochemistry or neurochemistry which seem to have some relationship to mental state, we can more reasonably restrict the discussion. In fact, if we limit the review to those areas which have *definitely* been shown to have a relationship to mental state, I could probably stop now. However, it may be worth while to examine some provocative leads and areas of activity.

Consciousness

There are four aspects of mental state: consciousness, memory, thought processes and affect or mood. With regard to consciousness, some years ago I had the opportunity to work with Carl Schmidt, who interested me in the cerebral circulation and in the energy metabolism of the brain. We developed a method for examining these functions in man and applied them to a variety of conditions. To generalize very broadly from much of the work that went on ten or twenty years ago in a number of laboratories, I think it is fair to say that the energy metabolism of the brain does appear to have a crude relationship to consciousness.[3] That is, when the metabolism of the brain is interfered with in some general and rather serious way, there is a depression of consciousness, so that if one examines a variety of patients in coma of different etiologic background, one finds in general that the energy utilized by the brain has fallen to 70 or even to 40 percent of its normal value. This depression in oxygen consumption is also correlated with the depth of the coma (Table 1).

There are a number of conditions, however, in which there does not appear to be a correlation between oxygen consumption and the mental state. These include such conditions as schizophrenia, the performance of mental arithmetic, or the psychosis produced by LSD (Table 2). In a number of other conditions, even though there are serious disturbances in consciousness, the oxygen consumption of the brain cannot be distinguished from that of normal cerebral function. In sleep the metabolism of the brain is also not different from normal. This is a finding which was very interesting to us, because the older idea of sleep, which had been generated by Sherrington and by Pavlov, equated sleep with coma or anesthesia — that is, that sleep represented a generalized depression of the activity of the brain. In fact Sherrington, in one of the most poetic passages of his book, *Man on His Nature,* describing the sleeping and waking brain, made an analogy to the city at night with all of the lights out. As morning comes, a light goes on here and another one there, until the whole city is ablaze with activity. This had been the thinking about the state of the nervous system in sleep. We were therefore very surprised to find that in the sleeping state, the oxygen consumption of the brain was quite normal[9] so that sleep was different from coma or anesthesia. Sleep appeared to be more of a shifting of the circuits of activity in the brain than any overall depression. As you are undoubtedly aware, there has been described in the past ten years a new phase of sleep called paradoxical or activated sleep, or sleep with rapid eye movements (REM sleep). This form of sleep occurs in animals and in man, where it appears to be associated with dreaming.

REM sleep has interested physiologists ever since it was described by Dement and by Jouvet[6] but we still know very little about it. It is still an ex-

Presented at the California Academy of Medicine meeting, St. Francis Hotel, San Francisco, Saturday, 2 Dec. 1967.

The author is Professor of Psychiatry, Harvard Medical School, Boston.

Reprint requests to: Department of Psychiatry, Harvard Medical School, Massachusetts General Hospital, Fruit Street, Boston, Mass. 02114.

TABLE 1.—*Reduced Cerebral Oxygen Consumption in Various States of Depression of Consciousness*

Condition	*Consumption Percent of Normal*
Senile psychosis	82
Diabetic acidosis	82
Insulin hypoglycemia	79
Artificial hypothermia	67
Surgical anesthesia	64
Insulin coma	58
Diabetic coma	52
Alcoholic coma	49

TABLE 2.—*Mental Conditions Not Correlated with Depressed Cerebral Oxygen Consumption*

Condition	*Consumption Percent of Normal or Control*
Normal sleep	97
Schizophrenia	100
LSD psychosis	101
Mental arithmetic	102
Anxiety	118
Epinephrine infusion	122

tremely mysterious phenomenon, but some of the things we have learned about it are remarkable. For example, in some recent studies with Evarts and Reivich, we have measured the regional blood flow of the brain in ordinary sleep and in rapid eye movement sleep, and have been thoroughly astonished to find that during the latter phase the blood flow to the brain is almost twice the normal value. There are very few conditions in which the metabolism of the brain is increased significantly, and practically nothing short of a convulsion will double the energy metabolism of the brain. Yet here is a phenomenon which is going on in all of us several times each night. Why this fantastic increase in blood flow during REM sleep occurs, I haven't the vaguest idea. I think it is probably in response to a comparable increase in oxygen utilization and metabolism, because other studies in California, by a group at UCLA,[2] have demonstrated a very sharp rise in brain temperature during REM sleep. This taken in association with the measurements of blood flow would be compatible with a pronounced increase in metabolism. This condition is still mysterious although we are learning a few more provocative things about it.

As everyone knows, there are many subtle things that go on in mental states that cannot be explained by the oxygen consumption alone. This is quite reasonable, for there are many things that happen to a radio or television set that cannot be explained by the power consumption. In the same way we would expect that other aspects of the chemistry of the brain would relate to more subtle aspects of mental state.

Memory

The next state that is worth mentioning, even if one does not engage in a long discussion, is memory. This has attracted the attention and interest of a number of scientists in the past five or six years, possibly because of the interesting findings of Hyden in Sweden about ten years ago.[8] He noted that in rats there were changes in the purine and pyridine base ratios of RNA in certain parts of the brain which appeared to correlate with learning. These data seem to have stood up in the hands of the few people who have tried to confirm them. From these interesting observations Hyden made a rather broad extrapolation to the hypothesis that just as genetic information is stored in the coding of the nucleotides in DNA, so the experimental information which the brain stores as memory was probably stored as sequences of amino acids or nucleotides in macromolecules in the brain. This hypothesis has attracted a great deal of attention and some support, although as a matter of fact I don't know of any firm evidence that supports it at the present time. There were some observations a year or two ago which suggested that when one trained rats to a particular task, one could then prepare an extract of the brain and inject it into other rats—naive rats —and in that way transmit the information. This, of course, if true, would have been very important evidence that memory was somehow stored as a chemical sequence in the macromolecule. Although the experiments have been repeated in many laboratories, in practically none has there been any evidence that a transfer of information actually took place. There are alternative explanations and none of them seem to support the idea that memory is literally transferred in this way.[10]

If we try to draw a generalization from much of the work in this field, I think the evidence does support the idea that protein synthesis may be crucial in the consolidation of memory — not so much in the acquisition of a learning experience, but in the ability of the brain to lay down that information into some permanent memory trace. I am referring now to a large number of experiments done by the Flexners, by Agranoff and by Barondes. They have used more or less specific inhibitors of protein synthesis injected into the brain of

a number of species at various stages during learning. Even though protein synthesis may be inhibited by 95 or 97 percent by some of these inhibitors, such as cyclohexamide or puromycin, the animal in most of these experiments continues to learn quite normally, but is unable to retain this information after several hours. I think it is a reasonable conclusion from much of this work that if one interferes with protein synthesis, one does in fact block the ability of the brain to consolidate memory.[1]

Now that, of course, is not proof that the memory is stored in the amino acid sequences of the protein, because the protein may be acting, and, in my own opinion, very probably is acting as a sort of solder, establishing new pathways or increasing the conductances of existing synapses. The memory may be stored in specific patterns in the network, the protein acting as a nonspecific solder necessary to establish these connections.

Thought Processes

If we move now to the third aspect of mental state, thought processes, I suppose the major disturbance of thinking which confronts us as physicians and especially confronts psychiatrists, is schizophrenia. This is a devastating disorder, and one in which thought processes seem to be involved. There has been a great deal of activity in the field of biochemistry as it relates to schizophrenia over the past ten or more years, in fact ever since physiological chemistry became a part of medicine and psychiatry. It is difficult to point with confidence to one area in all this activity which seems clearly to be leading to an understanding of the process of schizophrenia.[4] I don't mean to be negativistic or pessimistic, or to imply that this means that there is no biochemical aspect, but I cannot point to a particular area of biochemistry as being the most promising at the present time.

Let me mention some of the current hypotheses. Some 15 years ago a provocative hypothesis was developed by Osmond and Smythies, working with a biochemist in England, Harley-Mason. The interesting suggestion was made that the methyl group was probably involved in a number of biological processes that had to do with the catecholamines. It was predicted, in fact, that transmethylation, the adding of a methyl group, would turn out to be an important process in catecholamine metabolism. As a matter of fact, that prediction was confirmed more than ten years later by Axelrod, who showed, as I will indicate later, that methylation is one of the important ways in which epinephrine and norepinephrine are degraded in the body. Harley-Mason also pointed out, in conjunction with Smythies and Osmond, that a number of the psychotomimetic agents were methylated derivatives, especially mescaline, which was a very close analogue to dopamine, being essentially dopamine which had three methyl groups around the phenol ring. These men speculated that just as mescaline is an exogenous substance which produces a toxic psychosis, perhaps there was some abnormal metabolite of the catecholamines, a methylated metabolite, which produced the symptoms of schizophrenia, as a result of an internal toxin.

This is an interesting hypothesis, and in 1962 Pollin, Cardon and I, at the National Institute of Mental Health, had the occasion to test this hypothesis by giving methionine, which is a well recognized methyl donor and operates as such in many of the transmethylation processes in mammalian organisms. By giving large doses of methionine to some chronic schizophrenic persons, who had agreed to participate, and by giving a monamine oxidase inhibitor which would prevent the normal destruction of amines, we thought we would favor the conditions in which methylated amines could be produced, and see if they were produced in schizophrenic patients.

We also tested other hypotheses using other amino acids, and it was interesting that of some eight amino acids which we tested in this way, methionine was the only one that produced an intensification of the psychosis, and that in only a fraction of the schizophrenic subjects. Something like one-third of the patients whom we examined showed a decided intensification of psychosis with methionine. That was simply compatible with the transmethylation hypothesis; it by no means proved it. There are many reasons why methionine could be producing a toxic psychosis in schizophrenia, and we had by no means been sure that this was not a toxic psychosis superimposed on the schizophrenia. We have not been able, in fact, to convince ourselves that we can dissect that complex process from a true intensification of the schizophrenia *per se*. Nevertheless, the fact that methionine does intensify psychosis has been confirmed by a number of groups, and Himwich and his colleagues have shown that betaine, another methyl donor, will do the same thing. This lends further interesting support to the possibility that a trans-

methylation process has gone awry in schizophrenia.

Two years or so after those methionine experiments, Friedhoff reported finding in the urine of schizophrenic patients a substance which he identified as dimethoxyphenylethylamine (DMPEA). This is a remarkably interesting compound, and a number of us were very excited by this report because DMPEA is dopamine which is methoxylated in two positions as opposed to mescaline, the well-known hallucinogenic drug which is methoxylated in three positions. Thus DMPEA is two-thirds of the way toward mescaline; in fact it is the compound which Harley-Mason cited 12 years ago as being perhaps the most likely of the compounds that could be produced by transmethylation and cause schizophrenia!

This, of course, caused a great flurry of activity, and a number of laboratories rushed into the attempt to replicate Friedhoff's findings. The batting average varies from week to week. At the latest reading, many people have reported the finding of a "pink spot" in the urine of schizophrenic patients and not in the urine of normal persons. One of the best of these studies was done in Liverpool by Bourdillon, who took the precaution of having the examination of patients and urine done independently and blind. When he decoded the data, it was quite definitely demonstrated that in the patients who were clearly schizophrenic there was a high proportion in whom the pink spot was found. In those who were questionably schizophrenic a significant number yielded it, and in those who were not schizophrenic, the urine examination was negative with only one exception. The only difficulty is that Bourdillon did not control drug administration. The metabolites of the phenothiazines are notorious for persisting in the urine for weeks after the drug has been withdrawn, and for producing various colored spots in chromatograms of urine. As a matter of fact, there are two very convincing reports which have come from England in the past year in which the investigators seem to have proven quite well that the pink spot which is found in the urine of schizophrenic patients is not dimethoxyphenylethyamine at all. However, Friedhoff, in an analysis of gallons of urine from many patients with schizophenia, has isolated the material which he had previously identified chromatographically and, in a number of different assays demonstrated to his satisfaction that it is dimethoxyphenylethylamine. I think at the present time we cannot be sure, but the evidence is not conclusive that DMPEA excretion occurs in or is causally related to schizophrenia.

Another hypothesis in the etiology of schizophrenia was also advanced by Osmond and Smythies (later Hoffer joined them and Smythies left). It was postulated that in schizophrenia there was an abnormal metabolism of epinephrine which was transformed into a hallucinogenic agent, possibly adrenochrome. This hypothesis was widely promulgated for a number of years, to a large extent because it was difficult to test. No one knew anything about the normal metabolism of epinephrine, let alone its metabolism in schizophrenia. In order to test this hypothesis, we felt we needed some radioactive epinephrine of considerably greater activity than the C^{14}epinephrine which was then available. Epinephrine is such a powerful substance, and a safe dose is so small that a very high specific activity is required to carry out meaningful studies on its metabolic fate. This requirement was met with tritiated epinephrine of very high specific activity. With the help of this compound Axelrod very quickly worked out the metabolism of this amine. Now approximately 95 percent of its metabolic products can be accounted for (Chart 1). On the basis of this knowledge it was now possible to examine the metabolism of labeled epinephrine in schizophrenic patients. We found to our disappointment that the patients showed about the same patterns of metabolites of the administered epinephrine as did normal persons. No evidence of abnormal metabolites of catecholamines was found. Axelrod's work, however, prompted to some extent by the epinephrine hypothesis of schizophrenia, did lay the groundwork for knowledge of the metabolism of catecholamines in peripheral tissues and in the brain. This has been of immense subsequent usefulness.

Mood

I should like to turn now to neurochemical aspects of mood. This subject in my opinion is the most provocative and compelling area in the biochemical implications for psychiatry at the present time.[5] In a way this field can be said to have begun with the discovery of the psychotomimetic properties of lysergic acid diethylamide (LSD) more than 20 years ago. This substance, which has caused more than its share of social problems, has nonetheless been responsible for some important contributions to basic research, which we must not overlook. Next in the chain of seemingly unrelated

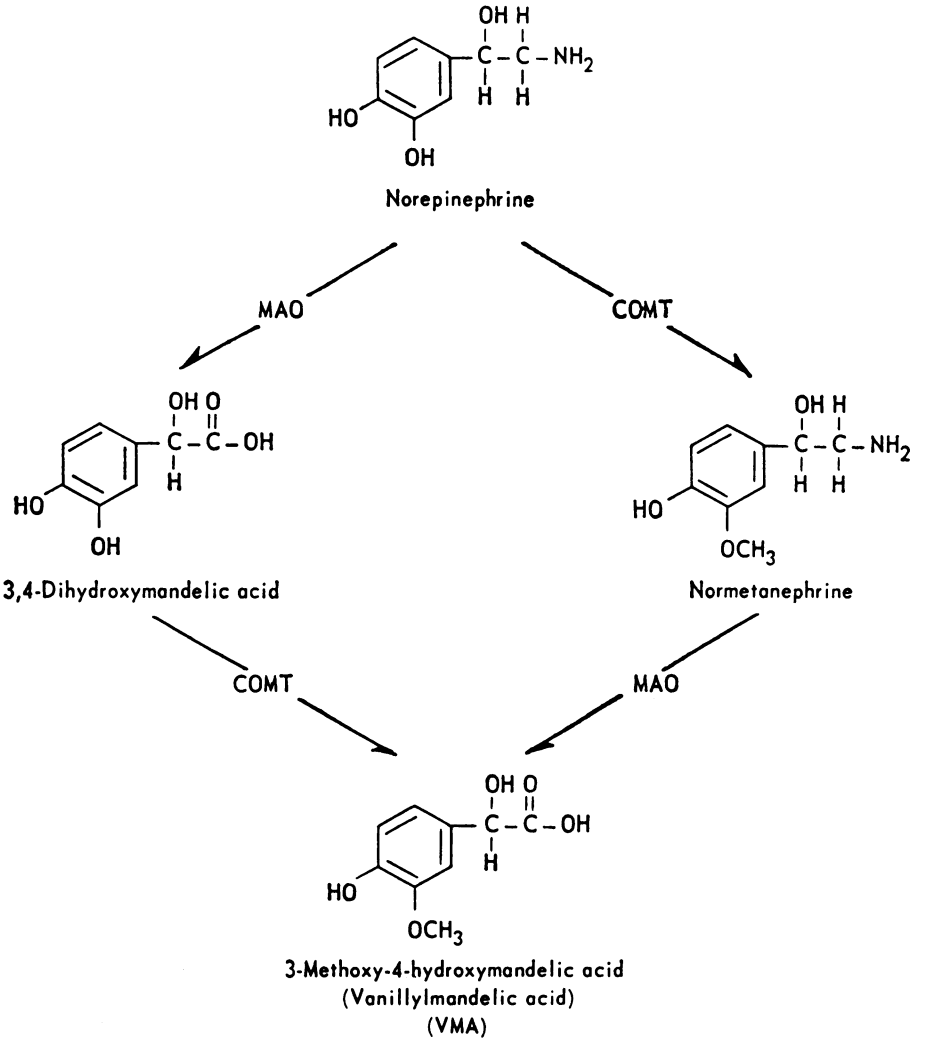

Chart 1.—Pathways of metabolism of norepinephrine (simplified). MAO, monoamine oxidase; COMT, catechol o-methyltransferase.

events was the discovery of a new biologically active amine by Rapport and Page in this country and by Erspamer in Italy, which the latter called enteramine and which in this country was called serotonin. It was found to be a simple indole amine, 5-hydroxytryptamine. Although highly concentrated in the gut, it was found in other parts of the body, especially in the brain. Here, it had a very interesting distribution. It was not uniformly dispersed, but was concentrated in the limbic system, in the hypothalamus, and in the brain stem that is, in the areas of the brain which neurophysiologists by that time had begun to suggest had something to do with emotional states. It was also known that serotonin affected smooth muscle. Then Gaddum in England and Wooley in this country made the very interesting finding that among the antagonists to serotonin, the best was LSD. This enabled both investigators to develop a rather interesting hypothesis that since LSD blocks the effect of serotonin on smooth muscle, and since serotonin is found in relatively high concentration in the brain, perhaps the reason that LSD produces psychosis is that it blocks the functions of serotonin in the brain. If that is the case, perhaps serotonin is necessary to keep us sane, and when something blocks, destroys or inhibits the serotonin in the brain, we become insane.

A few years later, iproniazid was introduced in the treatment of tuberculosis. It was found to be rather effective, as you remember, but it also produced a side effect of exhilaration. In fact, some of the first papers concerning the use of large amounts of this drug reported that the patients were dancing

in the halls of the sanitorium (and not because they had seen the x-ray report). They were dancing because iproniazid is a behaviorally activating drug. It was quickly learned that iproniazid was a monoamine oxidase inhibitor, and after its administration the concentration of serotonin in the brain rose appreciably. This was further evidence for Gaddum and Wooley's hypothesis.

There was also the finding that reserpine, the tranquilizing drug, also depleted serotonin in the brain. This further supported the hypothesis that serotonin played an important role in normal and abnormal mental states.

That simplistic explanation was very quickly challenged when shortly thereafter it was learned that a number of amines exist in the brain besides serotonin—norepinephrine and dopamine especially—and these amines are also affected by reserpine and iproniazid in much the same way as serotonin. It is fair to summarize a great deal of information by indicating that the activating effects of monoamine oxidase inhibitors and the depressant effects of reserpine and other congeners of reserpine appear to be related to the effects of these drugs upon one or more of these amines. We are not sure at the present time which specific amine is involved or whether all of them may not be involved in some very complicated way. However, the evidence since that time has tended to focus upon the norepinephrine in the brain. It exists there in high concentration, and there is considerable evidence that it is involved in mood and affect.

We have known since the time of Cannon that epinephrine has a great deal to do with the peripheral manifestations of anxiety, fear and rage. In recent years there has been an accumulation of evidence that central norepinephrine is equally involved in these affective states.[12]

Just before he died, Hillarp, the Swedish histochemist, developed a technique which was further pursued by his students, Dalhström and Fuxe, for demonstrating fairly specifically certain biogenic amines like norepinephrine, or serotonin in tissues. Their studies have revealed stores of norepinephrine in the vesicular nerve endings of certain neurons originating in the brain stem. The evidence is well established for particular neurons and tracts which seem to secrete norepinephrine, dopamine or serotonin. These neurons appear to have their cell bodies in the brain stem and to send axons throughout the brain, except for the dopamine-containing neurons which seem to be more localized to endings in the caudate nucleus.

A second technique which has contributed much to this field was devised by Gowinski and Axelrod, and is a means of labeling the norepinephrine in the brain by injecting tritiated norepinephrine into the cisterna magna or the lateral ventricle. These workers demonstrated that such labeled material quickly mixed with the endogenous pools of this amine in the brain, and could then be used to study its metabolism there.

As the result of work in the peripheral sympathetic nervous system, we can now picture a fairly good model of a sympathetic nerve ending. In these endings are a number of pre-synaptic vesicles which appear to contain norepinephrine. This overflows from the vesicles into the cytoplasm where it can be attacked by the monoamine oxidase which is in the mitochondria and is converted to the deaminated product. Some norepinephrine, and the functionally useful portion of it, eventually gets released from nerve endings into the synapse, where it acts on some ill-defined receptor in the postsynaptic cell and produces its effect. That norepinephrine can be O-methylated to form normetanephrine (NMN) or it can be taken up by some process which brings it back through the synaptic membranes into the axon (Chart 2).

To summarize considerable evidence, I think it is fair to say that every drug which has been found to affect mood, and which is useful in the treatment of depression, can be shown to have some action upon synaptic norepinephrine in the brain. For example, reserpine breaks the vesicular membranes in some way and releases the norepinephrine, but

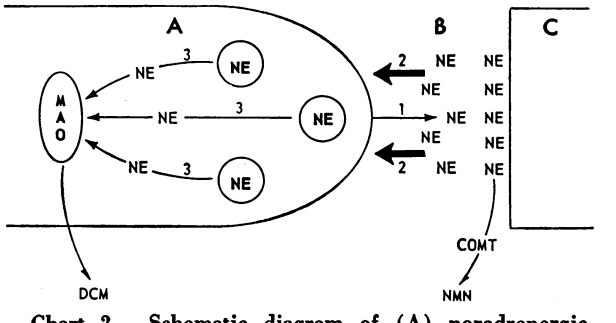

Chart 2.—Schematic diagram of (A) noradrenergic nerve ending, (B) synaptic cleft and (C) receptor. NE, norepinephrine; NMN, normetanephrine; DCM, deaminated catechol metabolites; COMT, catechol o-methyl transferase; MAO, monoamine oxidase (within a mitochondrion). 1, discharge of norepinephrine from nerve ending; 2, reuptake of norepinephrine from the synaptic cleft; 3, intracellular release of norepinephrine from vesicles to cytoplasm and to mitochondria. From Schildkraut and Kety.[12]

releases it presynaptically where it can be inactivated by the monoamine oxidase and released as a deaminated product. It never gets to the synapse and therefore diminishes the amount of effective norepinephrine. That may be why reserpine is a depressant agent. Monoamine oxidase inhibitors block that enzyme and increase the amount of norepinephrine available at the synapse. This can be inferred from the increase in normetanephrine which can be detected in the brain.

Amphetamine has a complex action, but among its effects are an inhibition of monoamine oxidase, a favoring of the release of norepinephrine at the synapse, an inhibition of its re-uptake, and perhaps an increased rate of synthesis, all of which would tend to increase the amount of norepinephrine acting at the synapse.

Finally, imipramine, which is very effective in the treatment of depression, doesn't block monoamine oxidase, and doesn't release norepinephrine, but has been shown recently to block the re-uptake of norepinephrine and therefore to increase the concentration of synaptic norepinephrine. Thus all these antidepressant drugs affect norepinephrine at central synapses in a way that would be compatible with the idea that this amine at the central synapses has something to do with mood.

Psychiatrists agree that electroshock is better than any of the drugs in the treatment of depression, yet there has never been a satisfactory explanation of how it acts. Last year at the Collège de France with Glowinski and a number of collaborators, we had an opportunity to tackle the problem of norepinephrine turnover in electroconvulsive shock.[7] We had found that in acute stress there is an increase in the turnover of norepinephrine in the brain, which we interpreted as indicating a greater release of the amine and a compensatory increase in its synthesis. We reasoned that since electroshock works for a prolonged period following the treatment, we would expect some persistent effect of electroshock upon norepinephrine synthesis in the brain. We did some experiments in which we subjected rats to electroconvulsive shock, twice a day for a week. When we examined the turnover of norepinephrine in the brains of these rats, 24 hours after the last shock, we found a 40 to 50 percent increase in turnover in the norepinephrine, suggesting a persistent augmentation in synthesis of this amine.

Thus electroconvulsive shock also takes its place with the anti-depressant drugs in favoring by one mechanism or another the availability of norepinephrine in the brain. This somehow lends credibility to the hypothesis that this amine in some way is involved in the depressive illnesses and in the beneficial effect of drugs and electroconvulsive shock. That is not to imply that it is the only amine involved or that depression can be explained by a decrease in brain norepinephrine. As in most psychiatric illness, the etiologic lineage is probably multifactorial and includes biological and genetic factors interacting with the powerful influences of life experience. But whatever the etiologic roots, it may be that in some of these illnesses biochemical factors form a crucial common path which can be changed to influence the clinical outcome.

REFERENCES

1. Agranoff, B. W.: Agents that block memory, *In* The Neurosciences, G. C. Quarton, et al.[11]

2. Kawamura, H., and Sawyer, C. H.: Elevation in brain temperature during paradoxical sleep, Science, 150, 912, 1965.

3. Kety, S. S.: Relationship between energy metabolism of the brain and functional activity, *In* Sleep and Altered States of Consciousness, S. S. Kety, et al.[6]

4. Kety, S. S.: Current biochemical approaches to schizophrenia, New Eng. J. Med., 276:325-331, 1967.

5. Kety, S. S.: The central physiological and pharmacological effects of the biogenic amines and their correlations with behavior, *In* The Neurosciences, G. C. Quarton, et al.[11]

6. Kety, S. S., Evarts, E. V., and Williams, H. L., Eds.: Sleep and Altered States of Consciousness, Res. Publ. Ass. Nerv. Ment. Dis., Vol. XLV, Williams and Wilkins Co., Baltimore, 1967.

7. Kety, S. S., Javoy, F., Thierry, A-M., Julou, L., and Glowinski, J.: A sustained effect of electroconvulsive shock on the turnover of norepinephrine in the central nervous system of the rat, Proc. Natl. Acad. Sci., 58:1249-1254, 1967.

8. Hyden, H.: Biochemical changes accompanying learning, *In* The Neurosciences, G. C. Quarton et al.[11]

9. Mangold, R., Sokoloff, L., Conner, E., Kleinerman, J., Therman, P. G., and Kety, S. S.: The effects of sleep and lack of sleep on the cerebral circulation and metabolism of normal young men, J. Clin. Invest. 34, 1092, 1955.

10. Quarton, G. C.: The enhancement of learning by drugs, and the transfer of learning by macromolecules, *In* The Neurosciences, G. C. Quarton, et al.[11]

11. Quarton, G. C., Melnechuk, T., and Schmitt, F. O.: The Neurosciences, Rockefeller University Press, N. Y., 1967.

12. Schildkraut, J. J., and Kety, S. S.: Biogenic amines and emotion, Science, 156:21-30, 1967.